Institute of Pharmaceutics and Biopharmaceutics[1], Martin-Luther-University Halle-Wittenberg, Halle/Saale and German Institute of Human Nutrition Potsdam Rehbrücke[2], Bergholz-Rehbrücke, Germany

Interactions between food components and drugs

Part 8: Effect of pectins and bile acid preparations forming stable mixed micelles on transport of quinine *in vitro*

B. Fritzsch[1], R. H. H. Neubert[1], G. Dongowski[2] and L. Heinevetter[2]

Interactions between quinine and acetylated pectin, amidated pectin and pectin with blockwise arrangement of the free carboxyl groups as well as interactions between quinine and bile salt preparations forming stable mixed bicelles have been investigated. A diffusion cell with two compartments and an artificial lipid membrane and a filter-grown colon carcinoma cell line (Caco-2) have been used. Depending on structural parameters, pectin preparations diminished the rate of permeation of the drug. Above the critical micelle concentration, the bile salt preparations influence the quinine transport stronger than the pectin preparations. The strongest inhibition of the quinine permeation showed a stable mixed micelle preparation consisting of glycodeoxycholate, palmitic acid and lecithin. The Caco-2 cell line appears to be not as suitable as artificial lipid membranes to study drug transport in the presence of the bile salt preparations.

1. Introduction

The bioavailability of orally applied drugs is influenced by food or food components [1–4]. Dietary fibres such as pectins are able to interact with drugs for a long time because they are not hydrolysed by the enzymes of the small intestine. Bile salts change the dissolution rate and the absorption of poorly absorbable drugs by lowering the surface tension of the gastrointestinal fluid or by micellar solubilization. They alter the tranport across the intestinal wall also by affecting the fluid and electrolyte balance. Bile salts decrease the viscoelasticity of the mucos layer, induce mucos secretion and influence the permeability of the mucosal membrane [5–16]. Therefore the aim of this study was to investigate the *in vitro* permeation of quinine across artificial lipid membranes in a two compartment diffusion cell in comparison with permeation across a filter-grown Caco-2 cell monolayer. The transport is influenced by the dietary fibre (pectin with different structural parameters) [17] and the bile salt (glycodeoxycholic acid and in combination with lineolic acid, palmitic acid, stearic acid, lecithin respectively) preparations forming stable mixed micelles. The objective of this work was to study *in vitro* the influence of dietary fibre and the bile salt preparations on the permeation of a lipophilic drug and to compare different model systems. For this purpose, the lipid membrane model of Neubert/Fürst (N/F-model) [20, 22] equipped with a dodecanol membrane and the Caco-2 cell line [23, 24] were used.

2. Investigations, results and discussion

In the N/F-model, the concentration of quinine increases generally in the acceptor compartment with icnreasing time. But in the presence of pectins (Table) with a blockwise arrangement of free COOH, the rate of permeation (flux) diminishs with decreasing the degree of esterification (Fig. 1). Additionally, the permeation of the drug decreases with an increasing degree of acetylation and amidation (Table) of the pectins (Fig. 1). However, these effects are relatively weak. A stronger influence on the flux of quinine has been observed in the presence of bile salt above the CMC. Addition of fatty acid or lecithin does not influence the transport of quinine in comparison

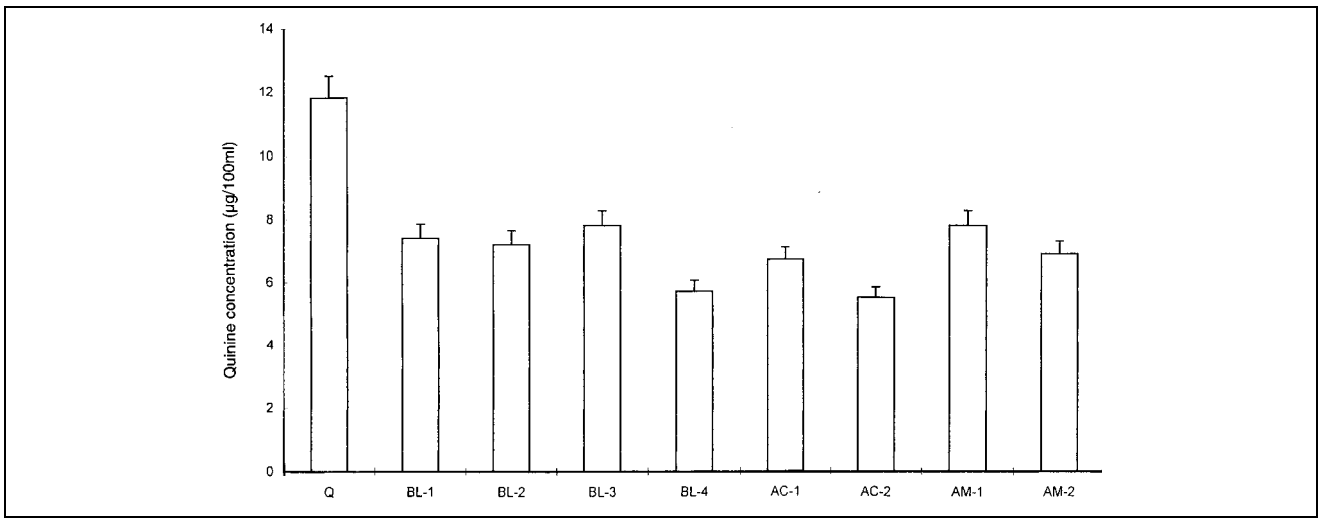

Fig. 1: Influence of pectins with a blockwise distribution of free carboxyl groups (BL), acetylated pectins (AC) and amidated pectins (AM) on the permeated concentration of quinine in the acceptor compartment (N/F model) after 120 min.
(Q = experiment with quinine in the absence of pectins)

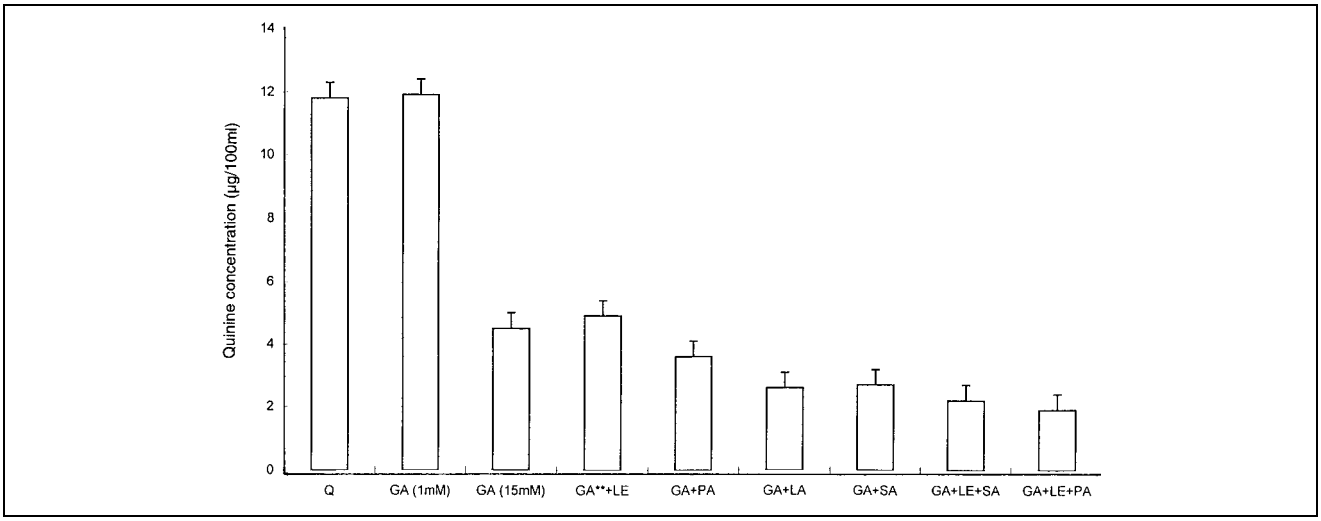

Fig. 2: Influence of glycodeoxycholic acid (GA) alone and in combination with lecithin (LE) and/or fatty acids on the permeated concentration of quinine in the acceptor compartment (N/F model) after 120 min
(Q = experiment with quinine in the absence of bile acids and micelles; GA^{**} = bile salt concentration: 15 mM; palmitic acid (PA), stearic acid (SA), linoleic acid (LA), and LE concentration = 1.8 mM).

with the bile salt preparation above the CMC. On the other hand, the combination of bilt salts and fatty acids or fatty acids and lecithin has the strongest influence on the transport of quinine (Fig. 2).

The mixtures of bile salts with fatty acids and/or lecithin are able to form stable mixed micelles [14, 15, 25, 26]. These stable micelles are formed during digestion of lipids in the small intestine. It is reported in the literature that drugs such as quinine have a very high affinity to these micelles due to ionic interactions between the anionic mixed micelles and the cationic quinine [21, 22, 32, 33]. Furthermore, hydrophobic interactions seem to take part in these interactions because quinine has a moderate lipophilicity at pH 7.2. Therefore, these stable mixed micelles are more important for potential interactions between drugs such as quinine and food constituents as discussed up to date in the literature. These results clearly indicate that the permetation of drugs such as quinine decreases much more rapidly by incorporation into stable mixed micelles than by interactions with dietary fibres such as pectins. The influence of the pectins on the permeation of quinine is lower despite of the existence of electrostatic forces between pectins and quinine. Dietary fibres such as pectins seem to have just a low potential for interactions with drugs. However, these results have to be confirmed *in vivo* at the pharmacokinetic level.

Table: Characterization of the pectins

Pectin preparation	Composition of the preparations			
	Galacturonan (%)	DE (%)	DAc (%)	DAm (%)
BL-1	72.8	92.8		
BL-2	72.7	71.8		
BL-3	89.8	54.4		
BL-4	73.9	34.5		
AC-1	75.5	49.2	13.5	
AC-2	84.5	48.6	55.0	
AM-1	72.0	39.3		10.3
AM-2	72.2	13.4		38.1

DE, degree of esterification; DAc, degree of acetylation; DAm, degree of amidation; BL, pectin with blockwise distribution of free COOH; AC, acetylated pectin; AM, amidated pectin

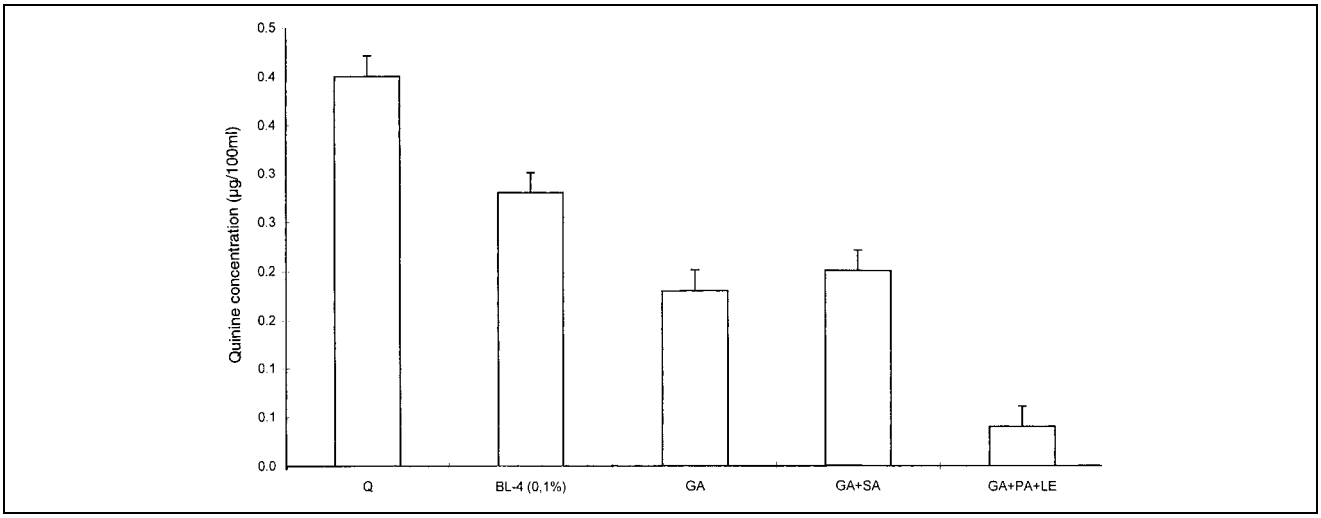

Fig. 3: Influence of pectin BL-4 or of glycodeoxycholic acid (GA) alone and in combination with stearic acid (SA) or palmitic acid (PA) and lecithin (LE) on the concentration of quinine in the acceptor compartment using Snapwell filter-grown membrane with Caco-2 cells after 120 min.
(Q = experiment with quinine in the absence of bile acids and micelles, concentrations see Fig. 2).

In the Cacao-2 cell monolayer system Snapwell™ the transport inhibition showed the same tendency. The presence of bile salts alone caused a concentration- and time-dependent loss in compact monolayer organsiation with disruption of cell contacts. The negative effect of the bile salts has been observed only when the bile salts were used alone. Nevertheless, the permeation of quinine into the acceptor compartment is similarly reduced compared to the results with the artificial lipid membrane model (Fig. 3).

The strongest effect on transport of quinine across the membranes is found with a ternary system consisting of glycodeoxycholic acid, palmitic acid and lecithin. The quinine concentration in the acceptor compartment is drastically reduced in this case.

Therefore, in the future it is necessary to investigate the influence of the incorporation of drugs such as quinine into stable mixed micelles on the pharmacokinetics of these drugs.

The transport across membranes and the resultant absorption of quinine is influenced by food components such as pectin or glycodeoxycholic acid preparations. In the present study, the results show a stronger decrease in absorption rate of quinine when mixed bile salt micelles are present in the donor solution. A bile salt concentration above the CMC shows the strongest interaction with quinine permeation, particularly in a mixture with high concentrations of fatty acid and lecithin. The use of a Caco-2 cell monolayer system does not yield more reliable results than the artificial lipid membrane because of a disruption of cell contacts of the monolayer organization when bile salts preparations were used.

In conclusion, the use of Caco-2 cell systems appears to be not very suitable to study gastrointestinal interactions caused by mixed bile salt micelles. Further studies with other model systems *in vitro* and *in vivo* are necessary to develop simple and rapid methods for detecting the interactions between drugs and food components. Based on the above results *in vitro* and *in vivo* experiments are in progress in order to evaluate the influence of the mentioned stable mixed micelles on the pharmacokinetics of quinine.

3. Experimental

3.1. Materials

The pectin solutions consists of 0.5% referred to galacturonan. Pectins with blockwise distribution of free carboxyl groups were prepared by gradually de-esterification of high-esterified pectin with pectin esterase from oranges [27]. Acetalyted pectins were prepared by treating pectin with acetic anhydride in formamide/pyridine. Amidated pectins were prepared with Nh_4OH in EtOH [16]. Glycodeoxycholic acid (Sigma-Aldrich, Deisenhofen, Germany) preparations in concentrations with exceeding and not exceeding the CMC and in addition with linoleic acid, palmitic acid and stearic acid (Sigma-Aldrich, Deisenhofen, Germany) and/or lecithin (Sigma-Aldrich, Deisenhofen, Germany) were taken.

3.2. Methods

The permeation model system of Neubert/Fürst (N/F-model) [20, 22] was used. It consists of donor and acceptor compartment separated by an artificial lipid membrane with dodecanol as lipid and collodium as matrix. The Snapwell™ diffusion chamber system was available with a filter-grown cell monolayer of human colonic carcinoma cell line Caco-2 (Costar, Bodenheim, Germany). Caco-2 cells were grown to confluency for 21 days on Costar snapwells under standard cell culture conditions. The experiments were carried out at pH 7.2 phosphate buffer (N/F) and culture medium (Snapwell TM) and 37 °C and at pH 7.2 in the presence of 1.1 mmol/l quinine (Caesar & Lorenz, Hilden, Germany). The concentration of quinine was measured in acceptor compartment using HPLC (Kontron, Neufahrn, Germany) with UV-detection.

* Part 7: Pharmazie **53**, 473 (1999) [34].

References

1 Neubert, R.; Fritzsch, B.; Dongowski, G.: Pharmazie **48**, 723 (1993)
2 Merkus, F. W. H. M.: Arzneimittel vor, während oder nach der Mahlzeit? 1. Aufl., S. 36, Wiss. Verlagsges. mbH, Stuttgart 1984
3 Pfeifer, S.: Pharmazie **48**, 3 (1993)
4 van Hoogdalem, E. J.; de Boer, A. G.; Breimer, D. D.: Pharm. Ther. **44**, 407 (1989)
5 Poelma, F. G. J.; Tukker, J. J.; Crommelin, D. J. A.: Acta Pharm. Technol. **36** (2), 43 (1990)
6 Borgström, B.; Barrowman, J. A.; Lindström, M.: Sterols and Bile acids p. 405, Elsevier Science Publishers B. V., Amsterdam 1985
7 Shiau, Y. F.: Physiology of the Gastrointestinal Tract 2nd Ed. p. 1527, Raven Press, New York 1987
8 Muranishi, S.: Pharm. Res. **2**, 108 (1985)
9 Ockner, R. K.; Isselbacher, K. J.: Rev. Physiol. Biochem. Pharmacol. **71**, 107 (1974)
10 Carey, M.; Small, D. M.: Am. J. Med. **49**, 590 (1970)
11 Carey, M. C.; Small, D. M.: Arch. Intern. Med. **130**, 506 (1972)
12 Fondacaro, J.: Proc. Soc. Exp. Biol. Med. **173**, 118 (1983)
13 Lindholm, A.; Henricsson, S.; Dahlqvist, R.: Br. J. Clin. Pharmacol. **29,** 541 (1996)
14 Scott-Moncrieff, J. C.; Shao, Z.; Mitra, A. K.: J. Pharm. Sci. **83**, 1465 (1994)
15 Gasco, M. R.; Trotta, M.; Eandi, M.: J. of Pharm. & Biomed. Anal. **2**, 425 (1984)
16 Luner, P. E.; Babu, S. R.; Radebaugh, G. W.: Pharm. Res. **11**, 1755 (1994)
17 Yang, S.; Bumgarner, J. G.; Kruk, L. F.; Khaledi, M. G.: J. Chromatogr. A **721**, 323 (1996)
18 Fricker, G.; Fahr, A.; Beglinger, C.; Kissel, T.; Reiter, G.; Drewe, J.: Br. J. Pharmacol. **117**, 217 (1996)
19 Schwarz, M.; Raith, K.; Rüttinger, H. H.; Dongowski, G.; Neubert, R.: J. Chromatogr. A **781**, 377 (1997)
20 Dongowski, G.; Schnorrenberger, B.; Plätzer, M.; Schwarz, M.; Neubert, R.: Int. J. Pharm. **158**, 99 (1997)
21 Schwarz, M.; Neubert, R.; Dongowski, G.: Pharm. Res. **8**, 1174 (1996)
22 Fritzsch, B.; Dongowski, G.; Neubert, R.: FASEB J. **8**, A 188 (1994)
23 Cullia, P. A.; Hope, M. J.: Nature **271**, 672 (1975)
24 Dangl, J. S.; Vyas, S. P.; Dixit, V. K.: Drug Dev. Ind. Pharm. **21**, 2021 (1995)
25 Howell, J. I.; Fischer, D.; Goodell, A. H.; Verrinder, M.; Lucy, J. A.: Bioch. Biophys. Acta **332**, 1 (1973)
26 Maggio, B.; Lucy, J. A.: Biochem. J. **156**, 353 (1976)
27 Muranushi, N.; Nakayima, Y.; Kinugawa, M.; Muranushi, S.; Sezaki, H.: Int. J. Pharm. **4**, 281 (1980)
28 Fürst, W.; Neubert, R.; Reppel, L.: Pharmazie **35**, 106 (1980)
29 Neubert, R.; Förtsch, R.; Beyrich, G.; Leisner, R.; Fürst, W.; Boltze, L.: Pharm. Acta Helv. **63**, 249 (1988)
30 Schneider, E.: BIOforum **4**, 132 (1996)
31 Artursson, P.: J. Pharm. Sci. **79**, 476 (1990)
32 Schwarz, M.; Neubert, R.; Rüttinger, H.: J. Chromatogr. A **745**, 135 (1996)
33 Schwarz, M.; Raith, K.; Dongowski, G.; Neubert, R.: J. Chromatogr. A **809**, 219 (1998)
34 Fritsch, B.; Neubert, R. H. H.; Dongowski, G.; Giessler, I.: Pharmazie **54**, 473 (1999)

Received March 29, 1999
Accepted July 15, 1999

Professor Dr. R. H. H. Neubert
Institute of Pharmaceutics
and Biopharmaceutics
Wolfgang-Langenbeck-Straße 4
D-06120 Halle/Saale
neubert@pharmazie.uni-halle.de